

Short Communication

Intraspecific Chloroplast DNA Variations of the Alpine Plants in Japan

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To obtain more information on the genetic differentiation of Japanese alpine plants, intraspecific variations of chloroplast DNA (cpDNA) of 23 species were investigated. We examined the samples from the populations in Central Honshu, Tohoku, and Hokkaido, Japan; and in Sakhalin, Russia. In the nucleotide sequence of two non-coding regions (*trnT-trnL-trnF* region and *rpl16* intron) of cpDNA, the intraspecific variations were detected in all species, except *Carex scita*. The species with a relatively high proportion of mutational events (including indels) were *Anemone narcissiflora* (0.90%), *Arenaria arctica* (0.74%), *Geum calthifolium* (0.86%), *Hypericum kamtschaticum* (1.04%), *Pedicularis yezoensis* (1.18%), and *Trollius riederianus* (0.77%).

Key words: chloroplast DNA, haplotype, intraspecific variation, Japanese alpine plants, phylogeography, *rpl16* intron, *trnT-trnL-trnF* region

In recent years, the intraspecific variations of cpDNA have been frequently used for inferring the evolutionary history or the past migration of plants (Avise 2000). In case of Japanese plants, several phylogeographical studies using cpDNA variations have been performed (*Abies*, Tsumura & Ohba 1994; *Polygonum*, Inamura *et al.* 2000; *Fagus*, Fujii *et al.* 2002; *Aucuba* and *Stachyurus*, Ohi *et al.* 2003a, b; *Michelia*, Lu *et al.* 2002; *Hemerocallis*, Noguchi *et al.* 2004; the plants of broad-leaved evergreen forests, Aoki *et al.* 2003, 2004; *Quercus*, Kanno *et al.* 2004). In Japanese alpine plants, geographic cpDNA variation among populations has been reported for several species (Fujii *et al.* 1995, 1996, 1997, 1999). Two or three major cpDNA clades were identified in Japan based on the results

of the phylogenetic analysis of the haplotypes in *Pedicularis chamissonis* Steven (Fujii *et al.* 1997) and *Primula cuneifolia* Ledeb. (Fujii *et al.* 1995, 1999). Furthermore, these clades have clearly shown the phylogeographical structure, and the clade endemic to the Central Honshu was observed in each species. The structure was discussed to be formed due to the glacial advances and retreats during the Pleistocene. In order to assess the generality of the distribution pattern of these clades, we must accumulate more data on cpDNA variations using other species. Fujii *et al.* (1996) investigated the intraspecific cpDNA variation of the other Japanese alpine plants using 40 taxa; however, the study has been limited with regard to the length of the nucleotide sequences of cpDNA (approximate-

ly 300–400 bp) and the number of populations analyzed. Therefore, in the present study, in order to obtain further information on the genetic differentiation of the alpine plants among populations in Japan, the intraspecific cpDNA variations were examined.

We selected 23 species of the subalpine or alpine plants that are commonly distributed in Japan (Appendix). The uncommon or rare species were avoided in order to obtain as many populations as possible. The species include the seven species on which a preliminary analysis was conducted in Fujii *et al.* (1996), as follows: *Arenaria arctica* (Caryophyllaceae), *Campanula lasiocarpa* (Campanulaceae), *Gentiana algida* (Gentianaceae), *Geum calthifolium* (Rosaceae), *Pedicularis yezoensis* (Scrophulariaceae), *Potentilla matsumurae* (Rosaceae), and *Trollius riederianus* (Ranunculaceae). A total of five to thirteen populations from Central Honshu, Tohoku, and Hokkaido districts of Japan; and the Sakhalin Island of Russia (Fig. 1, and Appendix) were examined for all the 23 species. During sampling, we collected relatively more populations from Central Honshu because high haplotype diversity was reported in previous studies (Fujii *et al.* 1997, 1999). A single individual from each population was analyzed. The taxonomy of all these species followed Shimizu (1982, 1983), though intraspecific taxa were not adopted in this study.

The leaves of the samples were dried and preserved in silica gel. The leaves were powdered using a mixer mill (Vibration Mill type MM300, Retsch, Haan, Germany), and total genomic DNA was extracted from 0.01 g of the powder by using the slightly modified CTAB method of Doyle & Dickson (1987) or a DNeasy Plant Mini Kit (Qiagen Inc., Hilden, Germany). The non-coding regions of cpDNA between the *trnL* (UAA) 5' exon and the *trnF* (GAA), including the *trnL* intron (Taberlet *et al.* 1991), and the *rpl16* intron (Jordan *et al.* 1996, Klechner & Clark 1997) were amplified using the polymerase chain reaction (PCR) method in most

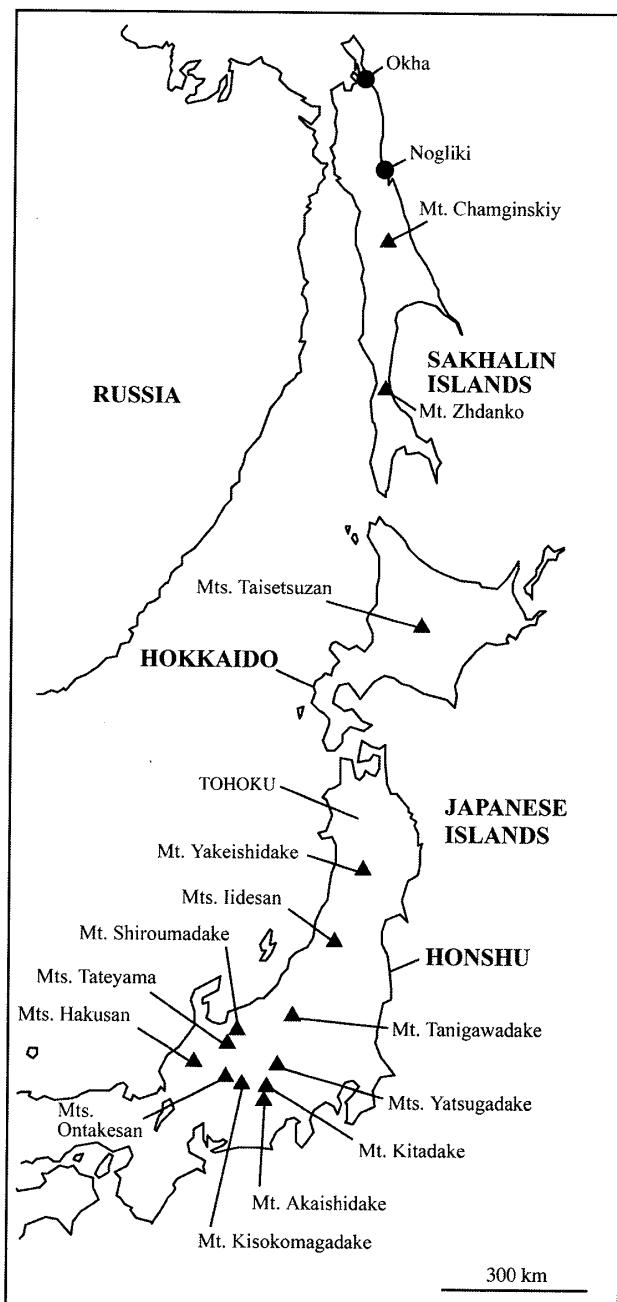


FIG. 1. The sample locations for the present study.

accessions. In *Arnica unalaschcensis* (Asteraceae), the non-coding regions between the *trnT* (UGU) and *trnL* 3' exon (Taberlet *et al.* 1991), and the *rpl16* intron were used. The non-coding regions between the *trnT* and *trnF* including the *trnL* intron (Taberlet *et al.* 1991) and the *rpl16* intron were used in the following four species: *Cassiope lycopodioides* (Ericaceae), *Gentiana algida*, *Geum calthifolium*, and *Pedicularis yezoensis* (Appendix). The

PCR products for direct sequencing were excised from 1% agarose gels and purified using a GeneClean III Kit (Qbiogene Inc., Carlsbad, CA, USA). Sequencing reactions were carried out using an ABI Prism BigDye terminator cycle sequencing ready reaction kit ver. 3.1 (Applied Biosystems Inc., Foster City, CA, USA). The sequencing reaction products were purified, concentrated by EtOH precipitation, and then applied to an ABI Prism 3100-*Avant* automated DNA sequencer (Applied Biosystems). The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank DNA databases under accession numbers AB219580 to AB219763. Alignment of the sequences was done manually using the DNASIS-Mac program ver. 3.0 (Hitachi Software Engineering, Tokyo, Japan). The insertions/deletions (indels) were generally placed so as to maximize the number of matching nucleotides in the corresponding sequences. Subsequently, we determined the cpDNA haplotypes of all the samples from site change and indel characteristics using the combined cpDNA regions. Additionally, we estimated the proportion of mutational events of each region in the following manner: the proportion of mutational events = (NS + ID)/L, where NS = number of nucleotide substitutions, ID = number of observed indels, and L = sequence length (O'Donnell 1992).

The non-coding region between the *trnL* 5' exon and *trnF* varied from 628 bp (*Hypericum kamtschaticum* (Clusiaceae)) to 1016 bp (*Carex stenantha* (Cyperaceae)) in length, whereas the *rpl16* intron varied from 752 bp (*Gentiana algida*) to 1338 bp (*Diapensia lapponica* (Diapensiaceae)) in length (Appendix). The *trnT-trnL* 3' exon in *Arnica unalaschcensis* was 1039 bp in length. The *trnT-trnF* region varied from 1446 bp (*Gentiana algida*) to 1747 bp (*Cassiope lycopodioides*) in length.

The proportion of mutational events (including indel characters) of each region was also variable depending on taxa (Appendix). The values varied

from 0% (*Carex scita* (Cyperaceae), *Diapensia lapponica*, and *Tilingia ajanensis* (Apiaceae)) to 1.59% (*Hypericum kamtschaticum*) in the *trnL-trnF* region, and from 0% (*Artemisia sinanensis*, *Cardamine nipponica* (Brassicaceae), *Carex scita*, *Cassiope lycopodioides*, *Luzula arcuata* (Juncaceae), and *Rumex montanus* (Polygonaceae)) to 1.83% (*Tilingia ajanensis*) in the *rpl16* intron region. The value of the *trnT-trnL* 3' exon in *Arnica unalaschcensis* was 0.48%. The values of the *trnT-trnF* region varied from 0.46% (*Geum calthifolium*) to 1.48% (*Pedicularis yezoensis*). We did not observe any correlation between the proportions of mutational events of the *trnL-trnF* region and the *rpl16* intron. For example, in *Cassiope lycopodioides*, although the value of the *trnL-trnF* region was relatively high, no intraspecific variation was observed in the *rpl16* intron. On the other hand, in *Tilingia ajanensis*, we observed the opposite pattern; the variation in the *rpl16* intron was relatively high, while that of the *trnL-F* region was 0%.

The proportion of mutational events combining the sequences of *trnT-L-F* regions and the *rpl16* intron ranged from 0% to 1.18% (Appendix). The species with relatively high values were *Anemone narcissiflora* (0.90%), *Arenaria arctica* (0.74%), *Geum calthifolium* (0.86%), *Hypericum kamtschaticum* (1.04%), *Pedicularis yezoensis* (1.18%), and *Trollius riederianus* (0.88%). On the other hand, the species *Artemisia sinanensis*, *Carex scita*, and *Luzula arcuata* indicated a value of 0.1% or lower.

Through the present investigations, three or more haplotypes were observed in most species (Appendix). For example, in *Cassiope lycopodioides* and *Geum calthifolium*, all populations analyzed in this study possessed different cpDNA haplotypes. Additionally, a relatively greater number of haplotypes were detected in the following species: *Anemone narcissiflora* (9 haplotypes), *Arenaria arctica* (7), *Cardamine nipponica* (7), *Diapensia lapponica* (7), *Gentiana algida* (8), *Loiseleuria*

procumbens (8), *Pedicularis yezoensis* (8), *Potentilla matsumurae* (8), and *Tilingia ajanensis* (7). On the other hand, only one or two cpDNA haplotypes were observed in *Artemisia sinanensis*, *Carex scita*, and *Luzula arcuata*.

According to the previous studies, in *Pedicularis chamissonis* (Fujii *et al.* 1997) the proportion of mutational events was 2.1% and 17 haplotypes were detected; and in *Primula cuneifolia* (Fujii *et al.* 1995, 1999) the proportion was 1.9% and eight haplotypes were recognized. In the present study, the values obtained for all these species were lower than those of the two above-mentioned species. *Pedicularis yezoensis* showed the highest value (1.18%) and the eight haplotypes were detected. However, we may be able to clarify the phylogenetic relationships among the cpDNA haplotypes by adding appropriate outgroups in the above-mentioned species that have a high proportion of mutational events and many haplotypes.

As mentioned above, the genetic differentiation between the populations of Central Honshu and northern area (Tohoku, Hokkaido, etc.) were inferred in *Pedicularis chamissonis* (Fujii *et al.* 1997) and *Primula cuneifolia* (Fujii *et al.* 1995, 1999). In the present analyses, among the populations of central Honshu and northern area, most species possessed different cpDNA haplotypes, excluding *Artemisia sinanensis*, *Carex scita*, *Hypericum kamtschaticum*, *Luzula arcuata*, and *Trollius riederianus* (Appendix). Although the present results are preliminary, these results might imply a similar situation as observed with *Pedicularis chamissonis* and *Primula cuneifolia*.

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APPENDIX. Intraspecific cpDNA variation of Japanese alpine plants.

Species and locality	Haplotype ¹ (<i>trnL-F</i>)	Haplotype ¹ (<i>rpl16</i> intron)	Combined haplotype	Voucher ²
<i>Anemone narcissiflora</i> L. (RANUNCULACEAE)				
Russia: Sakhalin, Mt. Zhdanko	1	1	A	N. Fujii F01060
Japan: Hokkaido Pref., Mts. Taisetsuzan	2 (1NS, 2ID)	2 (1NS)	B	K. Senni S1226
Japan: Iwate Pref., Mt. Yakeishidake	3 (1NS, 5ID)	1	C	N. Fujii F01703
Japan: Yamagata Pref., Mts. Iidesan	4 (3NS, 3ID)	3 (1NS)	D	N. Fujii F01787
Japan: Gunma Pref., Mt. Tanigawadake	-	3	-	N. Fujii F01744
Japan: Nagano Pref., Mt. Shiroumadake	5 (4NS, 3ID)	3	E	K. Senni S2617
Japan: Toyama Pref., Mts. Tateyama	4	3	D	N. Fujii F01652
Japan: Ishikawa Pref., Mts. Hakusan	5	3	E	K. Senni S874
Japan: Nagano Pref., Mts. Ontakesan	6	3	F	K. Senni S596
Japan: Nagano Pref., Mt. Kisokomagadake	7 (3NS, 5ID)	3	G	K. Senni S361
Japan: Yamanashi Pref., Mt. Kitadake	4	3	D	K. Senni S3225
Japan: Shizuoka Pref., Mt. Akaishidake	8 (3NS, 4ID)	3	H	K. Senni S1818
Japan: Nagano Pref., Mts. Yatsugadake	9 (4NS, 4ID)	3	I	K. Senni S2334
Length ³	950 bp	929 bp	1,879 bp	
Total polymorphic characters	8NS, 7ID	2NS	10NS, 7ID	
P ⁴ (%)	1.58	0.22	0.90	
<i>Arenaria arctica</i> Steven ex Ser. (CARYOPHYLLACEAE)				
Russia: Sakhalin, Mt. Zhdanko	1	1	A	N. Fujii F01070
Japan: Hokkaido Pref., Mts. Taisetsuzan	2 (1NS, 3ID)	2 (1NS, 2ID)	B	K. Senni S1552
Japan: Nagano Pref., Mt. Shiroumadake	2	3 (1ID)	C	K. Senni S2818
Japan: Toyama Pref., Mts. Tateyama	3 (1NS, 4ID)	4 (2NS, 1ID)	D	N. Fujii F01657
Japan: Nagano Pref., Mt. Kisokomagadake	4 (1NS, 4ID)	5 (1NS, 1ID)	E	K. Senni S243
Japan: Yamanashi Pref., Mt. Kitadake	2	3	C	K. Senni S3626
Japan: Shizuoka Pref., Mt. Akaishidake	2	6 (2NS, 3ID)	F	K. Senni S1998
Japan: Nagano Pref., Mts. Yatsugadake	5 (2NS, 4ID)	2	G	K. Senni S2354
Length ³	931 bp	970 bp	1,901 bp	
Total polymorphic characters	3NS, 4ID	5NS, 2ID	8NS, 6ID	
P ⁴ (%)	0.75	0.72	0.74	
<i>Arnica unalaschensis</i> Less. (ASTERACEAE)				
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	1	A	K. Senni S1392
Japan: Iwate Pref., Mt. Yakeishidake	2 (2NS, 2ID)	2 (3NS, 1ID)	B	N. Fujii F01705
Japan: Yamagata Pref., Mts. Iidesan	3 (1NS, 1ID)	3 (2NS, 1ID)	C	N. Fujii F01786
Japan: Gunma Pref., Mt. Tanigawadake	4 (1NS, 2ID)	4 (1NS)	D	N. Fujii F01740
Japan: Nagano Pref., Mt. Shiroumadake	5 (2NS, 1ID)	5 (4NS, 1ID)	E	K. Senni S3023
Japan: Toyama Pref., Mts. Tateyama	5	6 (3NS)	F	N. Fujii F01664
Japan: Nagano Pref., Mt. Kisokomagadake	5	6	F	K. Senni S157
Japan: Yamanashi Pref., Mt. Kitadake	5	6	F	K. Senni S3267
Japan: Shizuoka Pref., Mt. Akaishidake	5	-	-	K. Senni S1878
Length ³	1,039 bp (ad) ⁵	984 bp	2,023 bp	
Total polymorphic characters	3NS, 2ID	6NS, 2ID	9NS, 4ID	
P ⁴ (%)	0.48	0.81	0.64	
<i>Artemisia sinanensis</i> Y. Yabe (ASTERACEAE)				
Japan: Yamagata Pref., Mts. Iidesan	1	1	A	N. Fujii F01782
Japan: Nagano Pref., Mt. Shiroumadake	2 (1NS)	1	B	K. Senni S2940
Japan: Toyama Pref., Mts. Tateyama	2	1	B	N. Fujii F01656
Japan: Ishikawa Pref., Mts. Hakusan	2	1	B	K. Senni S1141
Japan: Nagano Pref., Mts. Ontakesan	2	1	B	K. Senni S557
Japan: Nagano Pref., Mt. Kisokomagadake	2	1	B	K. Senni S189
Japan: Yamanashi Pref., Mt. Kitadake	2	1	B	K. Senni S3366
Japan: Shizuoka Pref., Mt. Akaishidake	2	1	B	K. Senni S1718
Length ³	841 bp	972 bp	1,813 bp	
Total polymorphic characters	1NS	0	1NS	
P ⁴ (%)	0.12	0	0.06	

APPENDIX. Continued

Species and locality	Haplotype ¹ (<i>trnL-F</i>)	Haplotype ¹ (<i>rpl16</i> intron)	Combined haplotype	Voucher ²
<i>Campanula lasiocarpa</i> Cham. (CAMPANULACEAE)				
Russia: Sakhalin, Mt. Chamginsky	1	1	A	N. Fujii F01183
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	2 (1NS, 1ID)	B	K. Senni S1471
Japan: Nagano Pref., Mt. Shiromadake	2 (1NS)	3 (1NS, 1ID)	C	K. Senni S2656
Japan: Toyama Pref., Mts. Tateyama	2	3	C	N. Fujii F01655
Japan: Ishikawa Pref., Mts. Hakusan	2	3	C	K. Senni S1149
Japan: Nagano Pref., Mts. Ontakesan	2	3	C	K. Senni S780
Japan: Yamanashi Pref., Mt. Kitadake	2	3	C	K. Senni S3607
Japan: Shizuoka Pref., Mt. Akaishidake	2	3	C	K. Senni S2170
Length ³	913 bp	1,021 bp	1,934 bp	
Total polymorphic characters	1NS	2NS, 1ID	3NS, 1ID	
P ⁴ (%)	0.11	0.29	0.21	
<i>Cardamine nipponica</i> Franch. et Sav. (BRASSICACEAE)				
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	-	A	K. Senni S1352
Japan: Yamagata Pref., Mts. Iidesan	2 (2NS)	1	B	N. Fujii F01765
Japan: Nagano Pref., Mt. Shiromadake	3 (4NS)	1	C	K. Senni S2778
Japan: Toyama Pref., Mts. Tateyama	4 (3NS)	1	D	N. Fujii F01673
Japan: Ishikawa Pref., Mts. Hakusan	5 (5NS)	1	E	K. Senni S1020
Japan: Nagano Pref., Mts. Ontakesan	6 (5NS)	1	F	K. Senni S535
Japan: Nagano Pref., Mt. Kisokomagadake	3	1	C	K. Senni S42
Japan: Yamanashi Pref., Mt. Kitadake	3	1	C	K. Senni S3547
Japan: Shizuoka Pref., Mt. Akaishidake	3	1	C	K. Senni S1738
Japan: Nagano Pref., Mts. Yatsugadake	7 (3NS, 1ID)	1	G	K. Senni S2262
Length ³	872 bp	694+375 bp ⁶	1,941 bp	
Total polymorphic characters	8NS, 1ID	0	8NS, 1ID	
P ⁴ (%)	1.03	0	0.47	
<i>Carex hakkodensis</i> Franch. (CYPERACEAE)				
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	1	A	K. Senni S1694
Japan: Yamagata Pref., Mts. Iidesan	2 (5NS)	2 (2ID)	B	N. Fujii F01771
Japan: Nagano Pref., Mt. Shiromadake	3 (5NS)	3 (1ID)	C	K. Senni S3043
Japan: Toyama Pref., Mts. Tateyama	4 (4NS)	3	D	N. Fujii F01666
Japan: Ishikawa Pref., Mts. Hakusan	4	3	D	K. Senni S1098
Japan: Nagano Pref., Mts. Ontakesan	4	3	D	K. Senni S800
Japan: Nagano Pref., Mt. Kisokomagadake	4	3	D	K. Senni S452
Japan: Shizuoka Pref., Mt. Akaishidake	4	3	D	K. Senni S2108
Length ³	989 bp	849 bp	1,838 bp	
Total polymorphic characters	7NS	3ID	7NS, 3ID	
P ⁴ (%)	0.71	0.35	0.54	
<i>Carex scita</i> Maxim. (CYPERACEAE)				
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	1	A	K. Senni S1674
Japan: Nagano Pref., Mt. Shiromadake	1	1	A	K. Senni S2637
Japan: Toyama Pref., Mts. Tateyama	1	1	A	N. Fujii F01668
Japan: Ishikawa Pref., Mts. Hakusan	1	1	A	K. Senni S1133
Japan: Yamanashi Pref., Mt. Kitadake	1	1	A	K. Senni S3489
Japan: Nagano Pref., Mts. Yatsugadake	1	1	A	K. Senni S2548
Length ³	990 bp	848 bp	1,838 bp	
Total polymorphic characters	0	0	0	
P ⁴ (%)	0	0	0	

APPENDIX. Continued

Species and locality	Haplotype ¹ (<i>trnL-F</i>)	Haplotype ¹ (<i>rpl16</i> intron)	Combined haplotype	Voucher ²
<i>Carex stenantha</i> Franch. et Sav. (CYPERACEAE)				
Russia: Sakhalin, Mt. Zhdanko	-	1	A	N. Fujii F01086
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	2 (1ID)	B	K. Senni S1652
Japan: Nagano Pref., Mt. Shiroumadake	2 (2NS)	3 (1ID)	C	K. Senni S2837
Japan: Toyama Pref., Mts. Tateyama	2	3	C	N. Fujii F01646
Japan: Ishikawa Pref., Mts. Hakusan	2	3	C	K. Senni S1113
Japan: Nagano Pref., Mts. Ontakesan	-	4 (1NS, 1ID)	D	K. Senni S720
Japan: Nagano Pref., Mt. Kisokomagadake	3 (1NS)	3	E	K. Senni S266
Japan: Yamanashi Pref., Mt. Kitadake	3	3	E	K. Senni S3327
Japan: Shizuoka Pref., Mt. Akaishidake	3	3	E	K. Senni S2128
Japan: Nagano Pref., Mts. Yatsugadake	4 (1NS, 1ID)	3	F	K. Senni S2508
Length ³	1,016 bp	818 bp	1,834 bp	
Total polymorphic characters	2NS, 1ID	2NS, 1ID	4NS, 2ID	
P ⁴ (%)	0.30	0.37	0.33	
<i>Cassiope lycopodioides</i> (Pall.) D. Don (ERICACEAE)				
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	1	A	K. Senni S1208
Japan: Nagano Pref., Mt. Shiroumadake	2 (8NS, 2ID)	1	B	K. Senni S2704
Japan: Ishikawa Pref., Mts. Hakusan	3 (6NS, 2ID)	1	C	K. Senni S1170
Japan: Nagano Pref., Mts. Ontakesan	4 (6NS, 2ID)	1	D	K. Senni S576
Japan: Nagano Pref., Mt. Kisokomagadake	5 (5NS, 2ID)	1	E	K. Senni S318
Japan: Yamanashi Pref., Mt. Kitadake	6 (6NS, 2ID)	1	F	K. Senni S3567
Japan: Shizuoka Pref., Mt. Akaishidake	7 (7NS, 2ID)	1	G	K. Senni S2176
Japan: Nagano Pref., Mts. Yatsugadake	8 (6NS, 1ID)	1	H	K. Senni S2528
Length ³	1,747 bp (af) ⁵	884 bp	2,631 bp	
Total polymorphic characters	12NS, 3ID	0	12NS, 3ID	
P ⁴ (%)	0.86	0	0.57	
<i>Diapensia lapponica</i> L. (DIAPENSIACEAE)				
Russia: Sakhalin, Mt. Zhdanko (S-4)	1	1	A	N. Fujii F01061
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	2 (2ID)	B	K. Senni S1311
Japan: Iwate Pref., Mt. Yakeishidake	1	3 (1ID)	C	N. Fujii F01700
Japan: Yamagata Pref., Mts. Iidesan	1	2	B	N. Fujii F01768
Japan: Nagano Pref., Mt. Shiroumadake	1	4 (1NS, 2ID)	D	K. Senni S3063
Japan: Toyama Pref., Mts. Tateyama	1	4	D	N. Fujii F01651
Japan: Ishikawa Pref., Mts. Hakusan	1	5 (3NS, 2ID)	E	K. Senni S1186
Japan: Nagano Pref., Mts. Ontakesan	1	6 (1NS, 2ID)	F	K. Senni S515
Japan: Nagano Pref., Mt. Kisokomagadake	1	4	D	K. Senni S1
Japan: Yamanashi Pref., Mt. Kitadake	1	4	D	K. Senni S3528
Japan: Shizuoka Pref., Mt. Akaishidake	1	7 (2NS, 2ID)	G	K. Senni S2088
Length ³	920 bp	1,338 bp	2,258 bp	
Total polymorphic characters	0	3NS, 2ID	3NS, 2ID	
P ⁴ (%)	0	0.37	0.22	
<i>Gentiana algida</i> Pall. (GENTIANACEAE)				
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	1	A	K. Senni S1512
Japan: Nagano Pref., Mt. Shiroumadake	2 (3NS, 2ID)	2 (1NS)	B	K. Senni S2798
Japan: Toyama Pref., Mts. Tateyama	2	3 (2NS)	C	N. Fujii F01665
Japan: Nagano Pref., Mts. Ontakesan	3 (4NS, 2ID)	4 (2NS)	D	K. Senni S474
Japan: Nagano Pref., Mt. Kisokomagadake	4 (4NS, 1ID)	2	E	K. Senni S218
Japan: Yamanashi Pref., Mt. Kitadake	5 (3NS, 1ID)	5 (2NS)	F	K. Senni S3466
Japan: Shizuoka Pref., Mt. Akaishidake	5	2	G	K. Senni S2038
Japan: Nagano Pref., Mts. Yatsugadake	6 (3NS, 2ID)	2	H	K. Senni S2468
Length ³	1,446 bp(af) ⁵	752 bp	2,198 bp	
Total polymorphic characters	4NS, 3ID	4NS	8NS, 3ID	
P ⁴ (%)	0.48	0.53	0.50	

APPENDIX. Continued

Species and locality	Haplotype ¹ (<i>trnL-F</i>)	Haplotype ¹ (<i>rpl16</i> intron)	Combined haplotype	Voucher ²
<i>Geranium yesoense</i> Franch. et Sav. (GERANIACEAE)				
Japan: Iwate Pref., Mt. Yakeishidake	1	-	A	N. Fujii F01707
Japan: Yamagata Pref., Mts. Iidesan	1	-	A	N. Fujii F01779
Japan: Nagano Pref., Mt. Shiroumadake	2 (1NS, 1ID)	-	B	K. Senni S2960
Japan: Ishikawa Pref., Mts. Hakusan	3 (1ID)	-	C	K. Senni S896
Japan: Yamanashi Pref., Mt. Kitadake	3	-	C	K. Senni S3247
Japan: Shizuoka Pref., Mt. Akaishidake	3	-	C	K. Senni S1758
Length ³	926 bp	-	926 bp	
Total polymorphic characters	1NS, 1ID	-	1NS, 1ID	
P ⁴ (%)	0.22	-	0.22	
<i>Geum calthifolium</i> Sm. (ROSACEAE)				
Japan: Iwate Pref., Mt. Yakeishidake	1	1	A	N. Fujii F01702
Japan: Yamagata Pref., Mts. Iidesan	2 (4NS, 1ID)	2 (4NS, 5ID)	B	N. Fujii F01784
Japan: Gunma Pref., Mt. Tanigawadake	1	3 (5ID)	C	N. Fujii F01748
Japan: Nagano Pref., Mt. Shiroumadake	3 (1ID)	4 (4ID)	D	K. Senni S2857
Japan: Toyama Pref., Mts. Tateyama	4 (1ID)	5 (4NS, 5ID)	E	N. Fujii F01650
Japan: Ishikawa Pref., Mts. Hakusan	5 (1NS)	5	F	K. Senni S916
Japan: Nagano Pref., Mts. Ontakesan	-	6 (4ID)	G	K. Senni S659
Japan: Nagano Pref., Mt. Kisokomagadake	1	7 (1NS, 4ID)	H	K. Senni S136
Japan: Yamanashi Pref., Mt. Kitadake	6 (4NS)	8 (4NS, 5ID)	I	K. Senni S3347
Japan: Shizuoka Pref., Mt. Akaishidake	6	9 (4NS, 5ID)	J	K. Senni S1838
Japan: Nagano Pref., Mts. Yatsugadake	7 (4NS, 1ID)	2	K	K. Senni S2426
Length ³	1,738 bp(af) ⁵	1,063 bp	2,801 bp	
Total polymorphic characters	6NS, 2ID	9NS, 7ID	15NS, 9ID	
P ⁴ (%)	0.46	1.51	0.86	
<i>Hypericum kamtschaticum</i> Ledeb. (CLUSIACEAE)				
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	1	A	K. Senni S1187
Japan: Iwate Pref., Mt. Yakeishidake	2 (4NS, 5ID)	2 (2NS, 2ID)	B	N. Fujii F01715
Japan: Yamagata Pref., Mts. Iidesan	3 (5NS, 4ID)	2	C	N. Fujii F01777
Japan: Gunma Pref., Mt. Tanigawadake	3	2	C	N. Fujii F01742
Japan: Nagano Pref., Mt. Shiroumadake	4 (4NS, 3ID)	3 (2NS, 4ID)	D	K. Senni S2558
Japan: Toyama Pref., Mts. Tateyama	2	2	B	N. Fujii F01677
Japan: Ishikawa Pref., Mts. Hakusan	2	2	B	K. Senni S1040
Japan: Nagano Pref., Mts. Ontakesan	-	4 (3NS, 2ID)	-	K. Senni S740
Japan: Nagano Pref., Mt. Kisokomagadake	2	4	E	K. Senni S381
Japan: Yamanashi Pref., Mt. Kitadake	2	5 (2NS)	F	K. Senni S3187
Japan: Shizuoka Pref., Mt. Akaishidake	2	2	B	K. Senni S1898
Japan: Nagano Pref., Mts. Yatsugadake	-	2	-	K. Senni S2412
Length ³	628 bp	1,013 bp	1,641 bp	
Total polymorphic characters	5NS, 5ID	3NS, 4ID	8NS, 9ID	
P ⁴ (%)	1.59	0.70	1.04	
<i>Loiseleuria procumbens</i> (L.) Desv. (ERICACEAE)				
Russia: Sakhalin, 30km South of Okha	1	1	A	N. Fujii F01507
Japan: Hokkaido Pref., Mts. Taisetsuzan	2 (1ID)	2 (1ID)	B	K. Senni S1248
Japan: Yamagata Pref., Mts. Iidesan	3 (1ID)	3 (2ID)	C	N. Fujii F01790
Japan: Nagano Pref., Mt. Shiroumadake	4 (2NS, 2ID)	2	D	K. Senni S2706
Japan: Toyama Pref., Mts. Tateyama	5 (1NS, 1ID)	2	E	N. Fujii F01674
Japan: Nagano Pref., Mts. Ontakesan	5	2	E	K. Senni S454
Japan: Nagano Pref., Mt. Kisokomagadake	6 (2NS, 1ID)	4 (2ID)	F	K. Senni S64
Japan: Shizuoka Pref., Mt. Akaishidake	6	2	G	K. Senni S1918
Japan: Nagano Pref., Mts. Yatsugadake	7 (2NS, 1ID)	2	H	K. Senni S2223
Length ³	854 bp	956 bp	1,810 bp	
Total polymorphic characters	3NS, 2ID	2ID	3NS, 4ID	
P ⁴ (%)	0.59	0.21	0.39	

APPENDIX. Continued

Species and locality	Haplotype ¹ (<i>trnL-F</i>)	Haplotype ¹ (<i>rpl16</i> intron)	Combined haplotype	Voucher ²
<i>Luzula arcuata</i> (Wahlenb.) Sw. (JUNCACEAE)				
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	1	A	K. Senni S1573
Japan: Nagano Pref., Mt. Shiroumadake	2 (1NS)	1	B	K. Senni S2739
Japan: Toyama Pref., Mts. Tateyama	2	1	B	N. Fujii F01675
Japan: Nagano Pref., Mts. Ontakesan	2	1	B	K. Senni S639
Japan: Nagano Pref., Mt. Kisokomagadake	2	1	B	K. Senni S100
Japan: Yamanashi Pref., Mt. Kitadake	2	1	B	K. Senni S3386
Japan: Shizuoka Pref., Mt. Akaishidake	1	1	A	K. Senni S2048
Japan: Nagano Pref., Mts. Yatsugadake	2	1	B	K. Senni S2282
Length ³	848 bp	762+377 bp ⁶	1,987 bp	
Total polymorphic characters	1NS	0	1NS	
P ⁴ (%)	0.12	0	0.05	
<i>Pedicularis yezoensis</i> Maxim. (SCROPHULARIACEAE)				
Japan: Iwate Pref., Mt. Yakeishidake	1	1	A	no voucher specimen
Japan: Yamagata Pref., Mts. Iidesan	2 (4NS, 2ID)	1	B	N. Fujii F01788
Japan: Gunma Pref., Mt. Tanigawadake	3 (4NS, 5ID)	2 (3NS)	C	N. Fujii F01750
Japan: Nagano Pref., Mt. Shiroumadake	4 (7NS, 6ID)	3 (1NS)	D	K. Senni S2897
Japan: Toyama Pref., Mts. Tateyama	5 (8NS, 9ID)	4 (4NS)	E	N. Fujii F01661
Japan: Ishikawa Pref., Mts. Hakusan	6 (10NS, 6ID)	3	F	K. Senni S1177
Japan: Nagano Pref., Mt. Kisokomagadake	7 (7NS, 9ID)	5 (4NS)	G	K. Senni S178
Japan: Yamanashi Pref., Mt. Kitadake	7	5	G	K. Senni S3123
Japan: Shizuoka Pref., Mt. Akaishidake	7	5	G	K. Senni S1978
Japan: Nagano Pref., Mts. Yatsugadake	8 (6NS, 7ID)	5	H	K. Senni S2183
Length ³	1,627 bp (af) ⁵	831 bp	2,458 bp	
Total polymorphic characters	15NS, 9ID	5NS	20NS, 9ID	
P ⁴ (%)	1.48	0.60	1.18	
<i>Potentilla matsumurae</i> Th. Wolf (ROSACEAE)				
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	1	A	K. Senni S1452
Japan: Iwate Pref., Mt. Yakeishidake	2 (2ID)	2 (1NS, 1ID)	B	N. Fujii F01706
Japan: Yamagata Pref., Mts. Iidesan	2	1	C	N. Fujii F01794
Japan: Nagano Pref., Mt. Shiroumadake	3 (2ID)	3 (2NS, 2ID)	D	K. Senni S2980
Japan: Toyama Pref., Mts. Tateyama	3	4 (2ID)	E	N. Fujii F01659
Japan: Ishikawa Pref., Mts. Hakusan	3	5 (2NS, 1ID)	F	K. Senni S1000
Japan: Nagano Pref., Mts. Ontakesan	3	6 (2NS, 2ID)	G	K. Senni S701
Japan: Nagano Pref., Mt. Kisokomagadake	3	3	D	K. Senni S117
Japan: Yamanashi Pref., Mt. Kitadake	4 (1NS, 3ID)	7 (2NS, 4ID)	H	K. Senni S3307
Japan: Shizuoka Pref., Mt. Akaishidake	3	3	D	K. Senni S1938
Japan: Nagano Pref., Mts. Yatsugadake	3	5	F	K. Senni S2448
Length ³	978 bp	968 bp	1,946 bp	
Total polymorphic characters	1NS, 3ID	2NS, 5ID	3NS, 8ID	
P ⁴ (%)	0.41	0.72	0.57	
<i>Rumex montanus</i> Desf. (POLYGONACEAE)				
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	1	A	K. Senni S1413
Japan: Yamagata Pref., Mts. Iidesan	2 (1ID)	-	B	N. Fujii F01785
Japan: Nagano Pref., Mt. Shiroumadake	2	1	B	K. Senni S2577
Japan: Toyama Pref., Mts. Tateyama	3 (1NS, 1ID)	1	C	N. Fujii F01648
Japan: Nagano Pref., Mt. Kisokomagadake	4 (1ID)	1	D	K. Senni S387
Length ³	864 bp	691 bp (part) ⁶	1,564 bp	
Total polymorphic characters	1NS, 1ID	0	1NS, 1ID	
P ⁴ (%)	0.23	0	0.13	

APPENDIX. Continued

Species and locality	Haplotype ¹ (<i>trnL-F</i>)	Haplotype ¹ (<i>rpl16</i> intron)	Combined haplotype	Voucher ²
<i>Sieversia pentapetalum</i> (L.) Greene (ROSACEAE)				
Russia: Sakhalin, 30km South of Okha	1	1	A	N. Fujii F01505
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	2 (2ID)	B	K. Senni S1492
Japan: Iwate Pref., Mt. Yakeishidake	1	3 (1ID)	C	N. Fujii F01708
Japan: Yamagata Pref., Mts. Iidesan	1	4 (1NS, 4ID)	D	N. Fujii F01778
Japan: Gunma Pref., Mt. Tanigawadake	2 (1ID)	5 (3ID)	E	N. Fujii F01745
Japan: Nagano Pref., Mt. Shiroumadake	1	5	F	K. Senni S2684
Japan: Toyama Pref., Mts. Tateyama	-	5	-	N. Fujii F01649
Japan: Ishikawa Pref., Mts. Hakusan	1	5	F	K. Senni S960
Japan: Nagano Pref., Mts. Ontakesan	1	5	F	K. Senni S618
Japan: Nagano Pref., Mt. Kisokomagadake	1	5	F	K. Senni S19
Japan: Yamanashi Pref., Mt. Kitadake	1	4	D	K. Senni S3646
Japan: Shizuoka Pref., Mt. Akaishidake	1	4	D	K. Senni S1958
Length ³	996 bp	988 bp	1,984 bp	
Total polymorphic characters	1ID	1NS, 4ID	1NS, 5ID	
P ⁴ (%)	0.10	0.51	0.30	
<i>Tilingia ajanensis</i> Regel (APIACEAE)				
Russia: Sakhalin, 50km North of Nogliki	1	1	A	N. Fujii F01476
Japan: Hokkaido Pref., Mts. Taisetsuzan	1	2 (2NS, 1ID)	B	K. Senni S1372
Japan: Iwate Pref., Mt. Yakeishidake	1	3 (3NS, 1ID)	C	N. Fujii F01711
Japan: Yamagata Pref., Mts. Iidesan	1	4 (4NS, 1ID)	D	N. Fujii F01773
Japan: Gunma Pref., Mt. Tanigawadake	1	3	C	N. Fujii F01749
Japan: Nagano Pref., Mt. Shiroumadake	1	5 (6NS, 1ID)	E	K. Senni S2877
Japan: Toyama Pref., Mts. Tateyama	1	6 (4NS, 1ID)	F	N. Fujii F01654
Japan: Ishikawa Pref., Mts. Hakusan	1	7 (5NS, 1ID)	G	K. Senni S938
Japan: Nagano Pref., Mts. Ontakesan	1	7	G	K. Senni S816
Length ³	911 bp	930 bp	1,841 bp	
Total polymorphic characters	0	9NS, 2ID	9NS, 2ID	
P ⁴ (%)	0	1.83	0.60	
<i>Trollius riederianus</i> Fisch. et Mey. (RANUNCULACEAE)				
Russia: Sakhalin, Mt. Chamginskiy	1	-	A	N. Fujii F01170
Japan: Hokkaido Pref., Mts. Taisetsuzan	2 (5NS, 1ID)	1	B	K. Senni S1291
Japan: Iwate Pref., Mt. Yakeishidake	2	1	B	N. Fujii F01720
Japan: Yamagata Pref., Mts. Iidesan	2	1	B	N. Fujii F01802
Japan: Nagano Pref., Mt. Shiroumadake	2	1	B	K. Senni S2932
Japan: Toyama Pref., Mts. Tateyama	3 (5NS, 1ID)	2 (4NS, 1ID)	C	N. Fujii F01662
Japan: Nagano Pref., Mt. Kisokomagadake	4 (4NS, 3ID)	3 (3NS, 1ID)	D	K. Senni S427
Japan: Yamanashi Pref., Mt. Kitadake	5 (5NS, 3ID)	4 (3NS, 1ID)	E	K. Senni S3205
Japan: Shizuoka Pref., Mt. Akaishidake	2	1	B	K. Senni S1798
Japan: Nagano Pref., Mts. Yatsugadake	6 (5NS, 3ID)	5 (3NS, 1ID)	F	K. Senni S2393
Length ³	977 bp	961 bp	1,937 bp	
Total polymorphic characters	9NS, 3ID	4NS, 1ID	13NS, 4ID	
P ⁴ (%)	1.23	0.52	0.88	

¹ The number of variable site compared with Haplotype 1 are shown in parentheses; NS, Nucleotide substitution; ID, Insertion/Deletion. Dash (-) mean that we did not determine the nucleotide sequence of the region.

² The voucher specimens were deposited in the Makino Herbarium (MAK) of Tokyo Metropolitan University.

³ The length after multiple alignment analyses

⁴ The proportion of observed mutational events; NS+ID / sequence length (O'Donnell 1992).

⁵ ad: the non-coding region between *trnT* and *trnL* 3' exon, af: the non-coding region between *trnT* and *trnF*.

⁶ We did not determine the nucleotide sequence of a part of the target region because there were long poly-A or poly-T characteristics in that region.